Starch hydrolysis by a co-immobilized alpha-amylase and glucoamylase

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ABSTRCT

The industrial hydrolysis of starch involves several steps and several enzymes including, alpha-amylase (AA), glucoamylase (GluA), and pullulanase. Among the enzymes only GluA is used in immobilized form. There are several studies investigating immobilization of starch hydrolysis enzymes individually, however, only few studies exist on immobilization of multi-enzyme system.

The objective of this study is to develop a co-immobilized enzyme system for single step hydrolysis of starch to glucose. The immobilized enzyme system comprised of entrapped GluA inside the calcium alginate beads and adsorbed AA on the surface of the beads. The arrangement will provide the hydrolysis of starch into oligosaccharides on the surface followed by saccharification into glucose by glucoamylase entrapped inside the beads.

Co-immobilized enzyme system was prepared by dripping a mixture of alginate and glucoamylase into $CaCl_2$ solution followed by bringing the GluA containing beads into contact with alpha-amylase solution for adsorption. Starch hydrolysis reaction was performed at 55°C using 5mg/ml starch solution at pH 5.5. Glucose and starch concentrations in the reaction medium were monitored during reaction. The initial reaction rate was calculated from glucose concentration-time and starch concentration-time data.

The immobilization efficiencies for glucoamylase entrapment and alpha amylase adsorption were determined to be 45% and 54% respectively. Development of a co-immobilized enzyme system will improve the efficiency of starch hydrolysis process by reducing the number of steps in the process therefore reducing the enzyme and processing costs.

INTRODUCTION

Enzymes such as alpha-amylase as well as glucoamylase and pullulanase play very important roles in the starch hydrolysis. These enzyme account for ~$62 million annual spends in starch refinning industry [1]. Immobilization allows reusing of the enzyme, easy separation of the enzyme from the starch hydrolysis products, which can save enzyme, labor, and downstream costs [2]. The co-immobilization of enzyme may reduce the processing steps such as pH adjusting and ion exchange, it gives the potential to decrease the cost for the starch hydrolysis. The hypothesis of co-immobilized enzyme system is that: the alpha-amylase first degrade the starch by cutting the a-(1-4)-D-glucosidic bonds, then the smaller polysaccharide can diffuse inside the alginate beads , and react with gluco-amylase to produce glucose. Calcium alginate beads are widely used in enzyme immobilization due to the gel formation condition and no risk to human health.

OBJECTIVES

1. To determine the optimum pH for free AA and GluA,
2. To determine the optimum conditions for the preparation of co-immobilized enzyme system
3. To investigate the effect of immobilization on enzyme activity

METHODS & MATERIALS

Optimum pH for mixed GluA and AA free enzymes

The activity of GluA and AA were determined individually at 55 °C over a pH range of 4.5-7. Starch iodine test was utilized for AA activity and glucose test was used for GluA activity. The initial reaction rate was calculated from glucose concentration vs time or starch concentration vs time data at each pH studied.

Preparation of co-immobilized GluA (entrapment) and AA (adsorption) system

GluA and sodium alginate

↓ Drip by a peristaltic pump into $CaCl_2$ Incubate for 2 hours

GluA entrapped beads

↓ Add beads into AA solution

AA solution and GluA entrapped beads

↓ Incubate for 2 hours for adsorption of AA

Co-immobilized GluA and AA system

Determination of optimum immobilization conditions

The protein content of free enzyme solutions and the enzyme solutions after entrapment and adsorption steps were determined by Bradford method. This information was used to calculate the amount of GluA entrapped and the amount of AA adsorbed in the co-immobilized enzyme system.

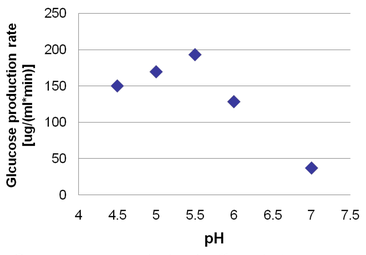
The entrapment efficiency and adsorption efficiency values were determined for various GluA and AA concentrations to find the optimum immobilization condition.

Starch hydrolysis by using co-immobilized enzyme system

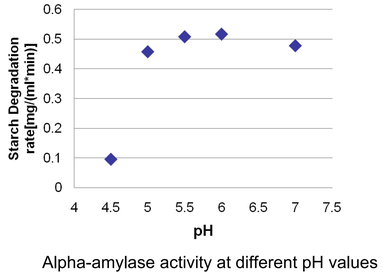
- 50 ml of starch solution (5mg/ml) at pH 5.5 was heated to 55°C in a batch reactor. 2 grams of alginate beads comprised of entrapped GluA and adsorbed AA were added to starch solution.
- 200 µl samples were removed from reaction medium every 1 minutes (up to 30 minutes) to monitor the starch and glucose concentrations in the reaction medium.
- Reaction was stopped by addition of 0.5N HCl prior to starch and glucose assays.
- Iodine test was employed to determine the starch concentration
- Glucose test kit (Sigma G3293) was utilized to determine glucose concentration.

RESULTS

Optimum pH of GluA and AA in solution



Glucoamylase acitivity at different pH values



Alpha-amylase activity at different pH values

Based on the individual enzyme studies, a pH of 5.5 was selected to be used for mixed enzyme system

RESULTS

Immobilization of GluA (entrapment) and AA (adsorption)

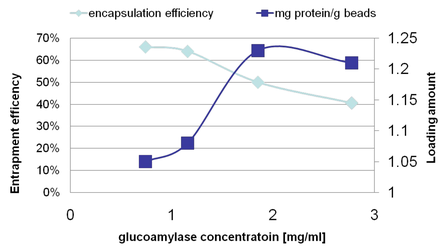


Figure The change of entrapment efficiency and loading amount vs. enzyme concentration

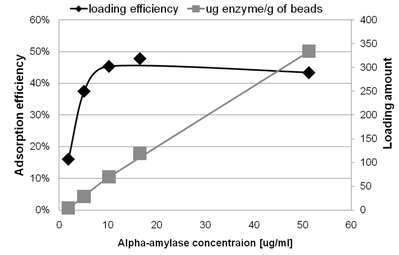
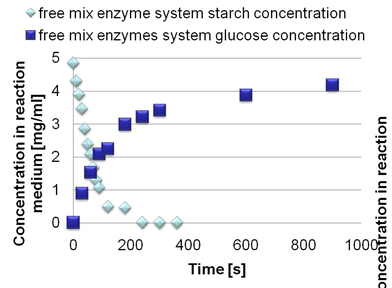
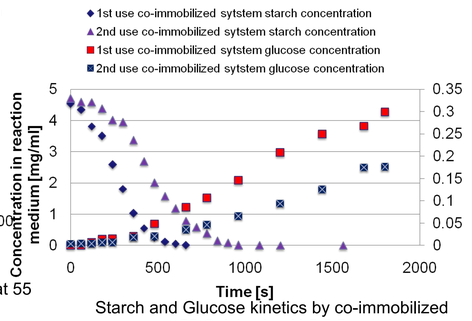


Figure The change of adsorption efficiency and loading amount vs. enzyme concentration

The hydrolysis kinetics of the co-immobilized system



Starch and Glucose kinetics by free enzyme at 55 Celsius in pH 5.5 5mg/ml starch



Starch and Glucose kinetics by co-immobilized enzyme at 55 Celsius in pH 5.5 5mg/ml starch

Different system	Starch degradation rate mg/(ml*min)	Glucose production rate ug/(ml*min)
Free mix enzyme	2.898	1459
1st use co-immobilized enzyme	0.702	4.152
2nd use co-immobilized enzyme	0.42	3.54

CONCLUSIONS

2. The optimum pH for the co-immobilized system studies was selected to be 5.5 based on the pH studies of individual enzymes in solution.
3. The amount of the enzyme (GluA and AA) in the co-immobilized system can be adjusted by changing the initial enzyme concentration during the immobilization process.
4. After the first use of co-immobilized enzyme system, no leakage of GluA was observed after 30 minutes of reaction time. However, there was a leakage of adsorbed AA.

REFERENCES

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2. Gerhartz (1990). Enzymes in industry. Production and applications.
3. Aniat et al., Alginate as Immobilization Material, Biotechnology, Vol.39, pp 186-194